IJIRR

International Journal of Information Research and Review
Vol. 05, Issue, 05, pp.5426-5429, May, 2018

RESEARCH ARTICLE

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF DROUGHT TOLERANCE GENE AND SCREENING OF DROUGHT TOLERANT RICE VARIETIES

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ARTICLE INFO

Article History:

Received 15th February, 2018
Received in revised form
09th March, 2018
Accepted 27th April, 2018
Published online 30th May, 2018

Keywords:

Drought, morphological,
Physiological,
Transcription factor

ABSTRACT

Rice is major staple food crop of world including India. Today with climate changes, all crops including rice expose to extreme water deficit condition, which adversely affects crop growth and yield. Drought is one of the most damaging abiotic stresses. Different plants response differently to drought stress. These responses include changes in gene expression. Abiotic stresses such as drought induced diverse physiological and molecular responses in plants. To determine physiological and molecular determinants of drought stress and screening of drought tolerant rice varieties, an experiment was conducted in a research station at Sardar Vallabh bhai Patel University of Agriculture and Technology, Meerut using thirteen rice varieties and two irrigation conditions (well watered and water stressed). The present study indicated that rice varieties N-22 and PD-11 are drought tolerant while IR-64 and PB-1 are drought sensitive rice variety. Drought tolerance of those varieties were measured based on rate of leaf rolling score. Leaf rolling score was positively correlated to chlorophyll content and proline accumulation. Significant increase in the proline content was also observed under drought stress. Out of thirteen rice varieties five showed the existence of OsLEA 30 gene under water stressed condition.

INTRODUCTION

Rice is one of the most important cereal crops in the world after maize and wheat. Rice is the world's single most important food crop and a primary food for more than a one-third of the world's population, mainly in the tropics. Worldwide, drought affects approximately 23 million hectare of rainfed rice. Abiotic stress is basically responsible for reducing average yields for most major crop plants to its half (Bray *et al*., 2000). Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants (Zhang *et al*., 2012). During drought stress water is removed from the membrane, it disturbs the normal bilayer structure and this makes the membrane abnormally porous when dehydrated.

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A significant number of genes, gene products and pathways associated with drought response have been identified in rice using a variety of experimental approaches (Gowda *et al*., 2004). Achieving drought resistance in rice will be necessary for meeting the growing water shortage of the world and it will require deeper understanding of the possible mechanisms available for drought resistance. Earlier regular random breeding approaches were used for creating new drought tolerant rice varieties but due to complex drought tolerance mechanism they were slow in progress (Ramya *et al*., 2010). Drought leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity through alterations in metabolism and gene expression. Control mechanisms for stress tolerance are based on the activation a regulation of specific stress related genes. So there is high research priority for breeding drought stress tolerance in crop plants by using transcriptome, proteome and metabolome (Wang *et al*., 2011). Numerous laboratory water stress experiments investigating dehydration induced changes in rice gene expression have revealed several candidate genes that may be associated with drought tolerance (Napolean *et al*., 2017).

LEA proteins play a special role in protecting cytoplasm from dehydration and storage of seeds and in whole plant stress resistance to drought (Lihua *et al*., 2017). The evolution of LEA proteins is one of these changes which play an important role in resistance to drought. This implies that studies on tolerant proteins, and the isolation, identification and functional analysis of their genes will be of great benefit to the breeding of drought resistant crops. Identification and characterization of drought tolerance gene for developing molecular marker and selecting genetic variation in plants are very useful (Pinto *et al*., 2010). The study demonstrate the potential value of newer technologies for identifying genes that might impart drought resistance and for using such genes to make crops more productive either in the presence or in the absence of drought stress (Chen *et al*., 2008) . The aim of this study is to identify and to the characterize drought tolerance LEA gene in rice varieties which tolerant, moderate and susceptible to drought. Developing rice plants resistance to drought is one of the famous methods to increase crop productivity. Nevertheless, this approach requires an understanding of physiological mechanisms at different developmental stage and duration of drought period. Hence, the aim of the present study is screening of drought tolerant rice varieties and to evaluate morphological, physiological, biochemical and molecular aspects of stress tolerance in different varieties of rice.

MATERIALS AND METHODS

Growth condition and Plant material: 13 rice varieties were utilized: PB-1, PB-6, N-22, IR-64, PD-6, PD-11, Govind, Saket-4, B-386, Swarna sub1, RB, PB-1121, and PB-1509. The experiment consisted of 2 treatments. Plants were grown in pots. Control plants were well-watered throughout the experiment; the drought stress treatment was conducted by irrigation holding after 80 days of germination.

Chlorophyll content (SPAD value): Chlorophyll content of leaf was determined by using SPAD meter. Leaf rolling score: Leaf rolling score was recorded mid day after stress using the scale described in Table 1, according to IRRI.

Proline determination: Free proline content was estimated following the procedure described by BATES et al. (1973). Youngest fully expanded leaves was frozen in the liquid nitrogen and stored in a refrigerator at - 70°C. 0.5g of frozen sample was ground with liquid nitrogen using pestle and mortar and homogenized with 10 ml of sulfosalicylic acid (3%). The homogenate was filtered using filter paper. 2 ml of the filtrate was added with 2 ml of glacial acetic acid and 2 ml acid ninhydrin in a test tube for one hour in a water bath at 95°C. The reaction mixture was then cooled in an ice bath for 10-15 minutes. After that, 4 ml of toluene was added to the reaction mixture and mixed vigorously with a test tube stirrer for 20 seconds. Supernatant layer was put into a quartz cuvette and measured at 520 nm using spectrophotometer to get the absorbance data. Toluene was used as blank. Proline standard curve was used to determine the concentration of proline in the samples.

DNA isolation

Total DNA was extracted from fresh rice leaf, using the method of Doyle and Doyle (1987). Fresh leaf with the weight of 0.2 gm was grinded with addition of liquid Nitrogen, and then 500 µl CTAB buffer was added and incubated for 30 minutes in water bath 65^{o}C. The DNA then was precipitated using 0.1 volume ammonium acetate and 2.5 volume ethanol absolute. The concentration and purity of extracted DNA was determined used spectrophotometric at the wavelength of 260 and 280 nm. Polymerase Chain Reaction: The total volume of PCR mixture was 20µl per-tube, which were consist of 11.9 µl dH_2O, 2 µl buffer Taq PCR; 1.6 µl $MgCl_2$; 1.6 µl dNTPs 2.5 mM, 0.3 µl primer forward-reverse, 0.3 µl Taq polymerase and 2 µl DNA. The PCR program was set on 93^{o}C for 1 minute preheating, continued with 30 cycles consisting of 1 minute Denaturation at a temperature of 93^{o}C, 1 minute annealing at a temperature of 57^{o}C, 1 minute extension at a temperature of 72^{o}C. A final extension was conducted for 1 minute at a temperature of 72^{o}C. The PCR product was visualized on 1% agarose gel. Agarose gel electrophoresis of PCR products Amplified PCR product was analyzed by 1.5% agarose gel electrophoresis. About 0.375g of agarose was melted in 25ml of 1X TBE buffer. The gel mixture was poured into a gel tray with comb at one end. After solidification, the agarose gel was removed carefully and kept in gel electrophoresis unit containing 1X TBE running buffer. 20µl of PCR product was loaded into the wells. The electrophoresis was carried out at 75 volt for about 40 minutes.

RESULTS

Leaf rolling score

Water stress significantly affected the leaf movement of rice. According to this experiment, N-22, PD-11 obtained the lowest leaf rolling score among the varieties while IR-64, PB-1 showed the highest leaf rolling score.

Table 1. Standard evaluation scale of drought tolerant rice

Scales	Description
	Leaf rolling
0	No symptoms (normal leaves)
1	Leaves starts folding (light V-shaped)
3	Leaves folding (deep V-shaped)
5	Leaves cupped fully (U-shaped)
7	Two leaf margins touching (O-shaped)
9	Leaves rolled tightly

Chlorophyll content (SPAD value): All the varieties showed higher chlorophyll content during well-watered condition compared with water stress except N-22 and PD-11 showed the highest chlorophyll content while IR-64 and PB-1 showed the lowest chlorophyll content during water stress (Table 2).

Proline content

The proline accumulation was higher under water stress condition compared with well watered condition in all the rice varieties (Table 2). Among the rice varieties, N-22 and PD-11 showed the highest increase in proline accumulation, and IR-64 and PB-1 showed the lowest value of proline activity during water stress.

Identification of LEA gene on rice

The OsLEA 30 gene detection of selected rice varieties under water stress and well watered was shown in Fig. 1. Amplification of LEA gene carrying of rice germplasm by OsLEA 30 showed the drought tolerance fragments as approx 190 bp.

Table 2. Chlorophyll content (mg.gfw^{-1}) and Proline content (µg g^{-1}) in rice varieties under well watered and water stressed condition

S.No.	Varieties	Chlorophyll content (mg.gfw^{-1})		Proline content (µg g^{-1})	
		Well watered	Water stressed	Well watered	Water stressed
1.	PB-1	32.06	8.16	0.077	0.070
2.	RB	27.83	10.16	0.074	0.092
3.	PB-1121	27.06	12.40	0.081	0.113
4.	PB-1509	30.36	10.36	0.096	0.124
5.	IR-64	27.66	7.23	0.071	0.064
6.	PD-6	30.13	9.60	0.078	0.144
7.	N-22	41.50	17.83	0.090	0.227
8.	PB-6	33.63	10.43	0.084	0.090
9.	Govind	30.70	10.33	0.100	0.103
10	PD-11	30.70	12.60	0.074	0.147
11.	Saket	29.46	9.23	0.087	0.107
12.	B-386	25.56	9.30	0.096	0.124
13.	Swarna	29.26	9.56	0.078	0.147
	Mean	30.45	10.55	0.083	0.119

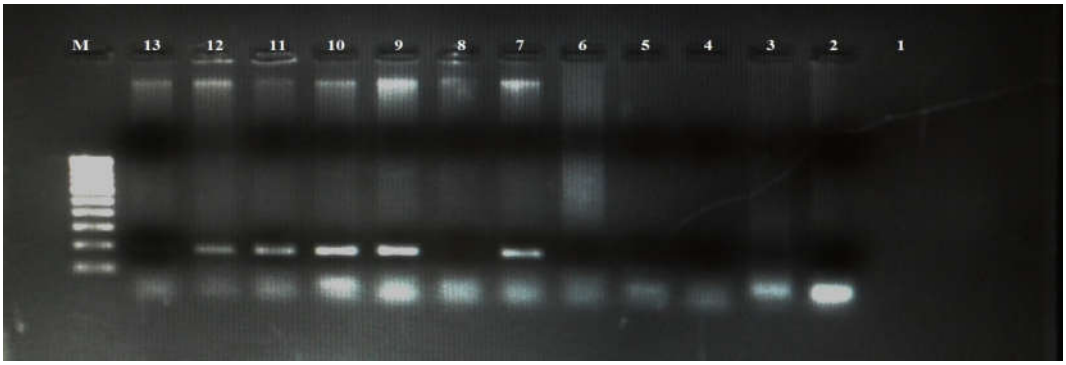

Fig 1. Amplification product of OsLEA 30 gene specific primer in 13 rice genotypes under non irrigated condition. Approximately 190 bp amplification product of Os LEA 30 gene indicated by an arrow M is 100 bp ladder

Five out of thirteen rice genotypes showed the appropriate amplification for drought tolerance fragments of LEA gene (N-22, Govind, PD-11, Saket, B-386) out of thirteen was successfully amplified. While, eight varieties (PB-1, RB, PB-1121, PB-1509, IR-64, PD-6, PB-6, Swarna) do not have LEA 30 gene. Drought did not alter LEA gene, this is indicated by the appearance of bands at 190 bp in drought condition. Basically, a gene provides the instructions for making a protein and proteins influence the characteristics of plants. Gene is genetic material which more stable than protein. Environmental stresses do not change the gene but may change the expression of the gene such as protein alteration. However gene variation can be induced by mutagenic agents such as radiation and certain chemicals.

DISCUSSION

The sensitivity of rice varieties to water stress was noted during our experiment. IR-64 and PB-1 were the most sensitive variety to water stress while N-22 and PD-11 was less sensitive. Leaf rolling score was positively correlated with drought score, chlorophyll content and proline accumulation. According to Chutia and Borah (2012), traditional rice varieties had long and droopy leaves with larger leaf angle, are more susceptible to rolling due to their ability to conserved water in plant tissue. Reduction of transpiration rate by creating microclimate is one of the benefits of leaf rolling (Kadioglu; Terzi, 2007; Kadioglu *et al*., 2012). It has been reported that greater leaf rolling may be an important indicator linked to drought tolerance and may have a positive impact on crop yield under water stress conditions. Proline contents in the leaf tissues increased significantly in all rice varieties except IR-64 and PB-1 (Table 2).

Proline acts as an important osmolyte that widely produced by plants to stabilize membranes and maintain the conformation of proteins at low leaf water potentials. Free proline accumulation is related to drought tolerance. Much drought tolerant plant has high accumulation of proline (Kadioglu; Terzi, 2007). LEA genes are a gene family plays important role in protection of water stress. *Os*LEA 30 genes is one of the members in LEA gene family. According to Wang *et al*. (2007), *Os*LEA 30 gene usually expressed under normal condition. However, this gene was expressed in five selected variety out of thirteen and thus conclusion on drought tolerance variety was not able to make from this result alone. Overall, the results obtained from the current study are helpful to elucidate physiological and molecular mechanism underlying the response of plants to drought stress, and discovery of genes for drought stress tolerance in rice. The present study indicated N-22 and PD-11 are drought tolerant while IR-64 and PB-1 were drought sensitive rice variety. Drought tolerant variety is selected based on rate of leaf rolling score, chlorophyll content and Proline content. These traits may have greater relevance and benefit to future breeding program, particularly for screening drought tolerance at early stage.

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